CHAPTER 9
CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF MOURINGA OLEIFERA PODS AND SILVER NANOPARTICLES SYNTHESIZED BY MOURINGA OLEIFERA PODS

9.1. INTRODUCTION

The fruits which hang down from the branches are three lobed pods and are 20-60cm in length. When they are dry they open into 3 parts, each pods contains 12-35 seeds [236]. Pods contain the polysaccharide d-galactose, 6-O-Me-D-galactose, D-galacturonic acid, l-arabinose, and l-rhamnose in a molar ratio of 1:1:1:1 and Nitriles, an isothiocyanate and thiocarbamates [260]. Pods can be boiled and eaten like beans. Fibre content in the pod increase when it grows and hence should be consumed when they can be easily broken [261].

The fruit of Moringa oleifera contains proteins, fats, carbohydrates, minerals, fibre, Vitamin A, ascorbic acid, tocopherol, oestrogenic substances [262].

This chapter deals with the structural and optical properties of MOP and its application as a green reducing agent in the synthesis process of silver nanoparticles and as an anti-microbial agent.

9.2. SYNTHESIS
9.2.1. Preparation Method of MOP Powder and Extract

Moringa oleifera pods were collected and were thoroughly washed thrice with double distilled water to remove dust particles, air-dried for a week under shade at room temperature. Then they were pounded to powder with metallic pestle and were stored in airtight containers for further analysis. To prepare the MOP extract, 5 gm of the pod powder was mixed well with 100 ml of double-distilled water and boiled at
60°C for 30 min. After boiling, the extract was filtered through Whatmann No.1 filter paper. The supernatant was collected and stored.

**9.2.2. Biosynthesis of Silver Nanoparticles by MOP**

1mM aqueous solution of silver nitrate (AgNO$_3$) was prepared and used as precursor to synthesise Ag nanoparticles. 1 ml of MOP extract was added to 100 ml of aqueous solution of 1 mM silver nitrate and kept at room temperature for 24 hours. The colour of the solution changed to reddish brown and the formed precipitate was washed with DD water, which was filtered and dried for two days in the sunlight.

**9.2.3. Synthesis Flowchart of Silver Nanoparticles using MOP**
9.3. STRUCTURAL CHARACTERIZATION

9.3.1. X-RAY DIFFRACTION ANALYSIS

Fig. 5.1a. XRD pattern of MOP

Fig. 9.1b. XRD pattern of silver nanoparticles synthesized by MOP
Fig. 9.1a shows the XRD pattern of MOP sample. Fig. 9.1b shows the XRD pattern of silver nanoparticles synthesized by MOP extract. The major peaks are obtained at 38.059°, 44.2462°, 64.3979°, 77.323°, 81.4555°, 97.8004° and the corresponding (hkl) values are (1 1 1), (2 0 0), (2 2 0), (3 1 1), (2 2 2) and (4 0 0) respectively. The obtained peaks are well matched with the standard data; JCPDS card no. 04-0783. The obtained silver nanoparticles are cubic in structure [83, 91, 184]. The values of cell parameters are calculated (a=b=c=4.084). The space group of the synthesized silver nanoparticles is Fm\(\bar{3}\)m(225).

The average crystalline size for the silver nanoparticles of this research was found from XRD data using Debye Scherer formula which relates the mean (volume average) crystallite size (D) of a powder to the broadening (\(\beta\)) of its powder diffraction peaks (ignoring other effects such as strain) and is given by,

\[ D_{hkl} = \frac{K\lambda}{\beta\cos\theta} \]  

(9.1)

where, \(D_{hkl}\) is the grain size, \(K\) is a dimensionless shape factor (0.94), \(\lambda\) is the wavelength of the X-ray, \(\beta\) is the line broadening at half the maximum intensity (FWHM), \(\theta\) is the Bragg’s angle.

Dislocation density is the measure of the number of dislocations in a unit volume of a crystalline material and which was found from the following equation.

\[ \text{Dislocation density (}\delta\text{)} = \frac{1}{D^2} \]  

(9.2)

where, \(D_{hkl}\) is the grain size.

The amount of deformation a material experiences due to an applied force is called strain. In practice, the magnitude of measured strain is very small, so it is often expressed as microstrain and which was found from following equation
\[ \text{Micro Strain (}\varepsilon\text{)} = \frac{(\beta \cos \theta)}{4} \quad (9.3) \]

where, \(\beta\) is the line broadening at half the maximum intensity (FWHM), \(\theta\) is the Bragg’s angle. The crystalline size, dislocation density and microstrain values were calculated and it is given in Table 9.1.

### Table 9.1. Structural parameters of silver nanoparticles synthesized by MOP

<table>
<thead>
<tr>
<th>2θ in (deg)</th>
<th>θ in (deg)</th>
<th>(d) spacing (Å)</th>
<th>(\beta) (deg)</th>
<th>(\beta)(rad)</th>
<th>Crystalline size (nm)</th>
<th>Dislocation (\times 10^{14}) lines/m²</th>
<th>Microstrain (10^{-3})</th>
</tr>
</thead>
<tbody>
<tr>
<td>38.06899</td>
<td>19.0345</td>
<td>2.359</td>
<td>0.2259</td>
<td>0.003943222</td>
<td>37.3625</td>
<td>7.16354</td>
<td>0.968995</td>
</tr>
<tr>
<td>44.24515</td>
<td>22.1226</td>
<td>2.044</td>
<td>0.2439</td>
<td>0.004257033</td>
<td>34.3141</td>
<td>8.49288</td>
<td>1.05508</td>
</tr>
<tr>
<td>64.29785</td>
<td>32.1489</td>
<td>1.445</td>
<td>0.2925</td>
<td>0.005104914</td>
<td>38.1716</td>
<td>6.86308</td>
<td>0.948457</td>
</tr>
<tr>
<td>77.31304</td>
<td>38.6565</td>
<td>1.231</td>
<td>0.3255</td>
<td>0.005681047</td>
<td>44.283</td>
<td>5.09947</td>
<td>0.817561</td>
</tr>
<tr>
<td>81.46551</td>
<td>40.7328</td>
<td>1.179</td>
<td>0.1972</td>
<td>0.003441266</td>
<td>42.3287</td>
<td>5.58123</td>
<td>0.855309</td>
</tr>
</tbody>
</table>

The average crystalline size is 39.2920 nm.

### 9.3.2. SEM Analysis

![SEM images of MOP](image1.png)

**Fig. 9.2. SEM images of MOP**
Fig. 9.3. SEM images of silver nanoparticles synthesized by MOP

Fig. 9.2 shows the SEM images of MOP sample. The particles of the MOP are not well dispersed and the aggregation of the particles is not seen.

Fig. 9.3 shows the SEM images of the silver nanoparticles synthesized by MOP. The SEM images reveal that the particles are well dispersed and the aggregation of the particles could be seen. The SEM images also revealed the spherical shape of the synthesized nanoparticles.

9.3.3. Energy Dispersive X-Ray Analysis (EDAX)

Fig. 9.4. EDAX spectrum of silver nanoparticles synthesized by MOP
Table 9.2. Elemental Analysis of silver nanoparticles synthesized by MOP

<table>
<thead>
<tr>
<th>Element</th>
<th>App Conc.</th>
<th>Intensity</th>
<th>Weight%</th>
<th>Weight%</th>
<th>Atomic%</th>
</tr>
</thead>
<tbody>
<tr>
<td>O K</td>
<td>3.45</td>
<td>0.3178</td>
<td>15.64</td>
<td>1.93</td>
<td>51.46</td>
</tr>
<tr>
<td>Si K</td>
<td>0.36</td>
<td>0.8339</td>
<td>0.62</td>
<td>0.15</td>
<td>1.16</td>
</tr>
<tr>
<td>Cl K</td>
<td>4.34</td>
<td>0.9541</td>
<td>6.55</td>
<td>0.28</td>
<td>9.73</td>
</tr>
<tr>
<td>Ag L</td>
<td>49.24</td>
<td>0.9188</td>
<td>77.19</td>
<td>1.79</td>
<td>37.66</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 9.4 shows the elemental profile of nanoparticles synthesized by MOP extract. The EDAX shows highest counts at 3 keV due to silver, which confirms the formation of silver nanoparticles.

9.4. OPTICAL CHARACTERIZATION

9.4.1. FTIR Spectral Analysis

9.4.1.1. FTIR Spectrum of MOP

Fig. 9.5a represents the FTIR spectra of MOL. The intense broad band at 3350.70 cm\(^{-1}\) is the characteristic of hydroxyl functional group in alcohols and phenolic compounds (isoquercetin, chlorogenic acid). The weak peak at 2916.39 cm\(^{-1}\) could be due to alkane C-H stretch which is associated with lipid molecule vibrations. The IR spectrum exhibited a peak at 2363.17 cm\(^{-1}\) for carboxylic acid [78]. The intense peak at 1654.90 cm\(^{-1}\) is associated with the amide groups in the protein and also it can be ascribed to aromatic C=C bending or NH of an amide [187]. A peak at 1244.90 cm\(^{-1}\) indicated the presence of - C=O (esters) and >C–O (ethers) in the compound. Peak at 1057.25 cm\(^{-1}\) is the empirical proof of identity for stretching vibration. The weak peak at 618.35 cm\(^{-1}\) is an evidence for metal – oxide vibration. Similar observations of metal oxides have been reported earlier [259].
9.4.1.2. FTIR Spectrum of Silver Nanoparticles Synthesized by MOP

Fig. 9.5b shows the IR peaks observed at 2924.08 cm\(^{-1}\), 2854.64 cm\(^{-1}\), 1577.77 cm\(^{-1}\) and 669.29 cm\(^{-1}\). The band at 2924.08 cm\(^{-1}\) is due to C-H asymmetric stretch whereas the band at 2854.64 cm\(^{-1}\) is due to C-H symmetric stretch. The absorption peak located at 1577.77 cm\(^{-1}\) may be attributed to secondary amine, NH bend. The peak found at 669.29 cm\(^{-1}\) means the presence of Thiol or thioether, CH\(_2\)-S- (C-S stretch) [186].
5.4.2. UV-Vis SPECTRAL ANALYSIS

Fig. 9.5b. FTIR spectrum of silver nanoparticles synthesized by MOP

Fig. 9.6a. Optical absorption spectrum of MOP and silver nanoparticles synthesized by MOP
Fig. 9.6a shows the UV-absorption spectrum of Ag nanoparticles synthesized by MOP. When the MOP extract was mixed in the aqueous solution of the silver ion complex, it started to change the colour from light green to dark brownish due to the reduction of silver ion, which may be the indication of the formation of Ag nanoparticles. Change of colour in aqueous solution of the silver ion complex is due to Surface Plasmon phenomenon.

From Fig 9.6b the band gap of silver nanoparticles synthesized by MOP was estimated to be 4.3179 eV. The increased value of the band gap may result due to the capping of bioorganic compounds on silver nanoparticles.

9.4.3. Photoluminescence Analysis

Fig. 9.7a shows the PL spectrum of the MOP. The relatively sharp emission peak centred at 529.98 nm is due to the free excitonic recombination between the top of the valence band and the bottom of the conduction band in saturated compounds. There is weak intensity broad emission within the spectral range of 280 to 400 and between
780 to 830 nm. This indicates the presence of a significant surface and volume defects in MOP powder [189, 190]. Structural defects inherent of MOP indicate its ability to enhance biological activity.

![Photoluminescence spectrum of MOP sample](image)

*Fig. 9.7a. Photoluminescence spectrum of MOP sample*

It was reported that silver nanoparticles display visible photoluminescence (PL) arising from interband transitions [182]. It was stated that silver nanoparticles synthesized from *P. chrysosporium* reported the photoluminescent emission peak observed at 423 nm. The intensity of the fluorescence varied directly with the absorption spectra and the position of fluorescence signal was found to be directly proportional to the nanoparticle size and surface functionalization [263].
Fig. 9.7b. Photoluminescence spectrum of synthesized Ag nanoparticles using MOP extract

Fig. 9.7b shows the photoluminescence spectra of silver nanoparticles synthesized by MOP. The PL emission has been obtained within the visible range, from 400 to 700 nm. The peak was obtained at 644.63 nm. The PL emission peak at 644.63 nm has been found to be red shifted from their corresponding UV-vis absorption peak. The visible luminescence of Ag nanoparticles is due to the excitation of electrons from occupied ‘d’ bands into states above the Fermi level. Subsequent relaxation by the electron-phonon scattering process leads to an energy loss and, finally, the photo luminescent radiative recombination of an electron from an occupied ‘sp’ band with the hole takes place. The optical properties of silver nanoparticles depend on both interband and intraband transitions between electronic states [264].
9.5. MAGNETIC CHARACTERIZATION

9.5.1. $^1$H NMR Analysis

The specific frequency report of $^1$H nucleus to its immediate vicinity is extremely sensitive to the electronic environment giving a type of identification number to an organic compound, called the NMR chemical shift. NMR chemical shifts provide immense information regarding structure and environment. Hence, NMR spectroscopy has become a powerful tool for the determination of organic structure [193].

Fig. 9.8. $^1$H NMR spectrum of MOP sample

Phytochemical screening of the extract of Moringa oleifera pods revealed the presence of various bioactive components of which alkaloid, phenolics, flavonoids, flavonols, proanthocyanidins, terpenoids, tannin, and cardiac glycosides are the most prominent [194]. Saponins were isolated from MOP and characterized [195].

Fig. 9.8 shows $^1$H NMR spectrum of present work which revealed a weak solvent (CDCl3) peak at 7.27 ppm and it is in accordance with the earlier study [195]. Fig. 9.8 shows the presence of saponins and Glycosides in MOP which was confirmed by the chemical shifts due to anomeric protons at δ5.33 and 5.06 ppm. This result well
matches with earlier reports [185]. Proton signals of downfield which shifts at δ1.94 ppm, 1.99 ppm, 2.00 ppm, 2.09 ppm and 2.18 ppm confirms the presence of phenolic compounds in MOP.

![Structure of Saponins in Moringa oleifera Pods](image)

**Fig. 9.9. Structure of Saponins in Moringa oleifera Pods**

### 9.7. ANALYSIS OF ANTIMICROBIAL ACTIVITY

#### 9.7.1. Antimicrobial Activity of MOP

![Antimicrobial activity of MOP against Corynebacterium diphtheria](image)

**Fig. 9.10a. Antimicrobial activity of MOP against Corynebacterium diphtheria**
Fig. 9.10b. Antimicrobial activity of MOP against *Pseudomonas aeruginosa*

In order to observe the antimicrobial activity of MOP extract, they are treated with pathogenic bacteria such as *Corynebacterium diphtheria* (gram positive bacteria) and *Pseudomonas aeruginosa* (gram negative bacteria) by Agar Well diffusion method.

The zone of inhibition around the well is shown in Fig. 9.10a and Fig. 9.10b for the two pathogens respectively. The effective inhibition of both gram positive and gram negative bacteria by MOP extract is of great significance as it demonstrates their broad-spectrum antimicrobial activity. No inhibition zone was observed for control, prepared by the stack solution taken in well without MOP extract and against *Corynebacterium diphtheria*. The antimicrobial activity of MOP extract should be associated with several mechanisms including generation of Reactive Oxygen Species (ROS) and hydroxyl radicals, release of ions from the MOP extract which simply penetrate into the cell wall and cause severe damage to the bacteria and kill them. Moreover, the anti-oxidants such as saponins and Glycosides were attached to the bacteria and disturb the usual function of bacteria and hence damage severely the outer surface of the bacteria such as DNA, lipids and proteins. Comparing the inhibition zones of both Fig. 9.10a and Fig. 9.10b it is found that MOP have robust antimicrobial activity on
*Pseudomonas aeruginosa* (gram negative bacteria) than *Corynebacterium diphtheria* (gram positive bacteria) bacteria. This greater antimicrobial activity against gram negative bacteria is ascribed to the variation in cell wall membrane of these bacteria. The outer membrane is permeable due to the presence of porins.

### 9.7.2. Antimicrobial Activity of Silver Nanoparticles Synthesized by MOP

![Antimicrobial activity of silver nanoparticles synthesized by MOP against *Corynebacterium diphtheria*](image1)

**Fig. 9.11a.** Antimicrobial activity of silver nanoparticles synthesized by MOP against *Corynebacterium diphtheria*

![Antimicrobial activity of silver nanoparticles synthesized by MOP against *Pseudomonas aeruginosa*](image2)

**Fig. 9.11b.** Antimicrobial activity of silver nanoparticles synthesized by MOP against *Pseudomonas aeruginosa*
Silver nanoparticles synthesized by MOP extract were studied for antimicrobial activity against *Corynebacterium diphtheria* (gram positive bacteria) and *Pseudomonas aeruginosa* (gram negative bacteria). The results for antimicrobial activity of silver nanoparticles are shown in Fig. 9.11a and Fig. 9.11b. Inhibition zone values were obtained from the synthesized silver nanoparticle suspension which were tested against *Corynebacterium diphtheria* (gram positive bacteria) and *Pseudomonas aeruginosa* (gram negative bacteria). The results and images of inhibition zones were presented in Fig. 9.11. The Ag nanoparticles synthesized showed inhibition zone against all the test organisms. Maximum zone of inhibition was found to be 12mm against *Corynebacterium diphtheria* (gram positive bacteria). From Fig. 9.11., it was found that the silver nanoparticles had better antimicrobial property towards gram positive bacteria as compared with gram negative bacteria.

Thus the Antimicrobial activity of silver nanoparticles synthesized by MOP against the bacteria is better than the MOP.

**9.8. CONCLUSION**

The structural characteristics of *Moringa oleifera* pods and its ability as a reducing agent for silver and its antimicrobial property were studied. XRD study confirms the formation of silver nanoparticles. The average crystalline size is 39.2920 nm. The surface morphology of the silver nanoparticles synthesized by MOP was analysed using SEM images. The SEM images revealed the spherical shape of the silver nanoparticles. EDAX confirmed the formation of silver nanoparticles. FTIR analysis helped to find the various functional groups present in the MOP and silver nanoparticles synthesized by MOP. The band gap of silver nanoparticles synthesized by MOP was estimated to be 4.3179 eV by Tauc plot. The Photoluminescence spectrum of silver nanoparticles synthesized by MOP had an emission peak at 644.63 nm which is
found to be red shifted from their corresponding UV-vis absorption peak. The \(^{1}\)H NMR spectrum of MOP sample reveals the presence of its organic constituents. Silver nanoparticles synthesized using MOP extract has shown enhanced antimicrobial activity on *Corynebacterium diphtheria* (gram positive bacteria) than *Pseudomonas aeruginosa* (gram negative bacteria).